Biological control of waterhyacinth in Sinaloa, Mexico with the weevils *Neochetina eichhorniae* and *N. bruchi* *

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Abstract. Water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) creates severe problems in the irrigation districts of Mexico, particularly in western Sinaloa. Therefore water hyacinth weevils (*Neochetina eichhorniae* Warner and *N. bruchi* Hustache), imported from the USA in 1993, were used to initiate a biological control program. Precautionary screening revealed that some were infected with a microsporidian so disease-free colonies were produced by eliminating infected breeding lines. To demonstrate effectiveness prior to open field releases, weevils were first released in cages at field sites. Weevil intensity increased to 6.3 weevils/plant after 320 days when the plants were all dead or dying. More than 8,600 *N. bruchi* and 14,500 *N. eichhorniae* were then released at various sites during January 1995 to August 1996. Waterhyacinth coverage declined at Batamote reservoir (134 ha) from 95% to <3% by 1997; at the 12-ha Hilda reservoir from 100% in May 1995 to 1% by March 1998; at the 42.3-ha Arroyo Prieto reservoir from 100% to 1% during the same interval; and at the Mariquita reservoir (492 ha), the largest reservoir in the Humaya system, from 394 ha (80%) to 98.4 ha (20%).

Key words: aquatic weeds (Pontederiaceae), biological control, Curculionidae, invasive plants, Mexico, water hyacinth

Introduction

Arreguín and Gutiérrez (1993) estimated that Mexico spends 240 million pesos (US \$24 million) annually to control 60 thousand hectares of weeds using chemical and mechanical methods. Aquatic weeds, which pre-empt

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efficient use of water resources by obstructing canals, clogging ditches, interfering with irrigation, and increasing evaporative losses, comprise the most severe weed problems. According to the Mexican National Water Commission (Comision Nacional del Agua, 1992), 25% of existing canals and 37% of drainage ditches are overgrown with aquatic weeds. Each irrigation district allocates 50 to 60% of its total budget to water resource conservation, 10% of which goes towards aquatic weed removal and control. It is therefore desirable to develop affordable, sustainable, environmentally benign, and long-term control methods. One such approach that has been recommended is biological control (Labrada et al., 1994).

Waterhyacinth (*Eichhornia crassipes*) is an aggressive aquatic weed in Mexican irrigation systems, occurring in 60 irrigation districts. The most severely affected are irrigation districts 010, Culiacan-Humaya-San Lorenzo, and 074, Mocorito, both located in the central coastal plains of the western state of Sinaloa. Sinaloa occupies a zone on the Pacific Coast between 22°22′ and 27°18′ N latitude. It straddles the Tropic of Cancer Tropic near Mazatlan. The coastal plain is warm and wet during the summer and mild during the winter. The absolute maximum and minimum temperatures at Mazatlán, for example, are 32.4 °C, and 11.0 °C, respectively. It receives an average annual rainfall of 803 mm, 82% of which falls during July to September, with the arid months of February to May receiving about 11 mm, on average (Müller, 1982). Irrigation District 010, Culiacán-Humaya-San Lorenzo was established by presidential decree during 1952.

Biological control agents that have been introduced into other countries for waterhyacinth control include the weevils Neochetina bruchi (Hustache) and N. eichhorniae Warner (Coleoptera: Curculionidae), the moths Niphograpta albiguttalis (Warren) and Xubida infusella (Walker) (Lepidoptera: Pyralidae), the mite Orthogalumna terebrantis Wallwork (Acarina: Galumnidae), and the bug *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae) (Julian and Griffiths, 1998). These agents slow plant growth and reduce water hyacinth densities and plant stature, possibly inducing lessened seed production (Center and Durden, 1986; Center, 1994; Center et al., 1990, 1999a, b; Julien et al., 1996). One of these insects, N. eichhorniae, was known from Vera Cruz State prior to 1967, apparently as an accidental introduction (O'Brien, 1976). However, neither it nor N. bruchi nor Niphograpta albiguttalis were found in Sinaloa during later surveys (Aguilar, pers. obs.; DeLoach, unpub. report¹). The Instituto Mexicano de Tecnologia del Agua (IMTA) and the United States Department of Agriculture (USDA) therefore cooperated to implement biological control agents, specifically the two Neochetina species and N. albiguttalis, for waterhyacinth control in the aforementioned irrigation districts. All three species of insects were collected

in Florida. However, a microsporidian disease of *Neochetina* spp. known from Florida (Rebelo and Center, 2001; Anonymous, 2001) dictated a cautious approach towards these introductions.

The importance of eliminating microsporidia from biological control agents prior to release has been debated (Kluge and Caldwell, 1992). Effects of microsporidiosis are typically sub-lethal, although they can become lethal when the host is stressed. They adversely affect many aspects of insect biology including pupal weight, adult longevity, developmental rates, adult fecundity, mating success, diapause, etc. (Steinhaus, 1954; Zimmack and Brindley, 1957; Kellen and Lindegren, 1973; Gaugler and Brooks, 1975; Andreadis, 1984; Tanada and Kaya, 1993). On the other hand, it has been argued that decontamination of stock is futile as agents often acquire similar diseases after release (see the discussion in Dunn and Andres, 1981). Furthermore, sanitization procedures involving the selection of a few "healthy" individuals from an already limited original stock could induce inbreeding depression thereby reducing the ability of the released population to establish or to control the plants. However, we ascribed to Dunn and Andres' (1981) recommendation that only disease-free individuals should be released, so precautions were implemented to prevent the concurrent introduction of a disease that might impair the effectiveness of the weevils or transfer to native organisms. Possibly due to this effort, the biological control program against waterhyacinth in Sinaloa, Mexico has been extremely effective.

Materials and methods

Colony development and disease elimination

The Irrigation and Drainage Technology Division of IMTA in cooperation with the Colegio de Postgraduados en Ciencias Agricolas (CPCA) evaluated the weevils *Neochetina bruchi* and *N. eichhorniae*. Permission was obtained during 1993 to import both species into Mexico so they were introduced from Florida, USA, during December of that year. However, microsporidiosis was detected by Dr. Ibarra (Centro de Investigación Avanzada, Irapuato, Guanajuato, México) during the initial quarantine so sanitation procedures were implemented. The microsporidian was known from Florida and has recently been determined to be an undescribed species by Dr. James Becnel at the USDA/ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida. Recent work, including scanning electron microscopy and DNA analysis indicates that more than one species might be involved and definitive studies are continuing (Rebelo and Center, 2001; Anonymous, 2001).

Weevils were reared at the CPCA near Mexico City. Microsporidia can be transmitted transovarially from adults to offspring, so it was necessary to eliminate infected breeding lines. Disease-free lines were developed by placing individual pairs of weevils (622 pairs of *N. eichhorniae* and 54 pairs of *N. bruchi*) in separate cages with host plants. The parent weevils were checked for entomopathogens 3–4 months later. The progeny and host plants were eliminated if disease organisms were evident in either parent. If the parent weevils were healthy, then plants containing their progeny were added to cultures. Releases were initiated when sufficient quantities of disease-free individuals had been produced, but the sanitization procedure continued until March 1995, after the first releases had begun.

Pupae (1,200 total) collected from roots provided virgin adults. The cocoons were submersed in a 1% CuCl solution for 5 min. They were then suspended in small cups within a loosely compacted piece of synthetic cotton above a 1 cm layer of natural cotton that had been saturated with 1% CuCl, the cups were covered with a fine mesh cloth that was secured with an elastic band. The cups were placed in egg cartons that, in turn, were enclosed in black plastic bags. The bags were held in an incubator at 25 °C, 80% RH, and 16:8 L:D photoperiod. Emerged adults, removed weekly, were fed pieces of waterhyacinth leaves.

The 107 adults subsequently obtained were held in isolation until they could be checked for disease. A frass sample from each was examined for microsporidial spores one week after they emerged. Infected individuals were eliminated. This procedure was repeated every few weeks. Initially, 17 (16%) of 107 weevils were infected. These breeding lines were eliminated and the subsequent generation was checked. No pathogens were detected from 36 pairs, so these were incorporated into the general colony. This procedure was repeated with a second group during November and December 1994. Microsporidiosis was detected in 21 (6%) out of 339 individuals. These were eliminated which provided 138 "healthy" pairs of N. bruchi and 10 healthy pairs of N. eichhorniae. The subsequent generation was examined during January 1995 when microsporidiosis was detected in one pair of N. eichhorniae and one pair of N. bruchi and three pairs were infected by a bacterium. These were eliminated. Ultimately, 3,500 pupae were collected and reared, yielding 455 adults. No diseased individuals were found out of 391 examined. These formed the basis for a greenhouse colony that supported later field releases.

Weevils were collected quarterly from each site after release until December 2000. On each occasion, 15 adults were examined for microsporidial infection. Diagnoses were done by the technical staff of the Universidad Autónoma de Sinaloa, Culicán, Sinaloa, México.

Field cage demonstration

A field demonstration of the effectiveness of the weevils was required by local interests prior to initiation of open releases. Six floating cages (2 m wide \times 2 m deep \times 1 m tall) made from 10 cm diameter (i.e., standard 4 in.) polyvinyl chloride (PVC) pipe frames were constructed and placed within a homogeneous waterhyacinth mat at a reservoir (Dique 8) in Sinaloa. Diseasefree weevils were transported in plastic vials (3 cm diam., 8.5 cm length), which were partially filled with moist wood shavings. These were placed in an ice chest and moved from CPCA to Sinaloa by air transport. Thirty-six pairs of both weevil species (72 total: 20 N. bruchi and 52 N. eichhorniae) were released on 25 Oct. 1994 into each of two cages but none were placed in a single screened control cage or in an unscreened control plot. A second release was made on 25 Jan. 1995 of 45 pairs in one cage and 83 pairs in the second. Evaluations were done intermittently during the following year when the live plants in each cage were counted. Fifteen plants from each cage were randomly selected during each evaluation to measure growth and development of the insect populations and the condition of the plants. The length of the third leaf (generally the youngest fully mature leaf) of each plant was measured and the total number of leaves per plant was counted. A detailed examination was made of 15 plants in the treatment cages to count adults, larvae, and pupae and quantity of feeding scars on the third position leaf. Likewise, the waterhyacinth leaf density in a 0.50 m \times 0.50 m (0.25 m²) quadrat, representative of the enclosed mat, was determined in triplicate within each cage. Five plants were randomly harvested from each cage during the last examination (day 320) to measure live and dead biomass.

Open releases

Open releases of weevils were made at water bodies containing major infestations of water hyacinth beginning in January 1995. A total of 23,137 insects were released between January 1995 and August 1996 (8,612 *Neochetina bruchi* and 14,525 *N. eichhorniae*) at 41 locations within 18 water bodies. Only 500 were liberated in irrigation district 063, Guasave, and 500 in district 075, Los Mochis, the remainder were released in districts 010 and 074, Culiacán. Fifteen plants were randomly sampled at each of two or three locations within each water body to determine the growth and expansion of the insect populations and their impacts on water hyacinth. Sampling was done weekly and the same parameters were evaluated as in the cage experiment. However, additional data included standing crop (g fresh wgt/m²), plant density (number/m²), and leaf measurements (petiole length and length and width of leaf blades on the third youngest leaf). These data were obtained

Table 1. Counts of *Neochetina* spp. adults, larvae, and pupae on plants in caged plots provisioned with the weevils for the field cage demonstration trial

Time	Infestation intensity (counts/plant) ¹						
(days)	Adults		Larvae	Pupae			
	NB	NE	NB & NE	NB & NE			
1	0.10	0.26	0.0	0.0			
90	0.03	0.86	0.13	0.13			
201	1.06	0.86	8.13	2.27			
320	2.93	3.40	1.20	0.67			

¹NB = *Neochetina bruchi*; NE = *N. eichhorniae*; Means based on 15 plants.

from three or four (depending on the variability of the plant mats) 1 m² quadrat samples. Plant density and standing crop data were used in conjunction with surface coverage determinations to estimate the total number of plants and the total plant weight (biomass) at each impoundment.

Results and discussion

Colony development and disease elimination

The extensive effort to eliminate pathogens from the stock colonies was apparently effective. Despite regular and continued quarterly monitoring at each site, microsporidia were never detected in field-collected weevils.

Field cage demonstration

The initial insect density in these cages (Day 1) averaged only 0.36 adults/plant but increased to 6.3 adults/plant 320 days after release (Table 1). The larval population peaked prior to this at about 8 larvae/plant. At this level, the damage was intense and had severely impacted all of the plants by the end of the trial.

Measures of the status and the condition of the plants demonstrated that reductions were due to the deterioration and subsequent death of the plants. All parameters measured showed the same trends but results were best reflected by the biomass data (Table 2). Total plant weight (which included dead tissue) was reduced by caging the plants but it was not greatly affected by the presence of weevils. However, the biomass of living plant tissue in cages with weevils was reduced to about half of that in cages without weevils. As a result, dead tissue accounted for about 41% of the total plant weight

Table 2. The weights of waterhyacinth plants in the field cage demonstration trial compared between caged (with or without weevils) or uncaged plots (no weevils) after 320 days

	Biomass of 5 plants $(n = 2)^1$				
Treatment	Total wgt. (g)	Live wgt. (g)	Dead wgt. (g)		
Cage + weevils	2700b (±200)	1600c (±100)	1100a (±100)		
Cage, no weevils	4050b (±250)	3000b (±100)	$1050a~(\pm 150)$		
No cage, no weevils	5900a (±300)	$4350a\ (\pm 150)$	1550a (± 150)		
Anova F(p)	40.2 (0.007)	133.5 (0.001)	4.1 (0.137)		

¹Means followed by the same letter are not significantly different (p = 0.05) based upon the All Pairwise Multiple Comparison Procedure (Tukey Test). Values in parentheses represent Standard Errors of the Means. F-values are derived from 1-way Analyses of Variance (Anova).

in cages with weevils as compared to 26% in cages without weevils or in uncaged control plots. Plants in the cages with insects all appeared to be dying by the end of the study whereas those in cages without insects or in uncaged control plots were all alive and healthy.

Open releases

The liberation of 23,137 weevils (8,612 *Neochetina bruchi* and 14,525 *N. eichhorniae*) between January 1995 and August 1996 substantially reduced infestations of waterhyacinth within Irrigation Districts 010 and 074, Culiacan, Sinaloa (Table 3). This reduction greatly improved the operation of the hydrological network. This improvement was confirmed through interviews with agricultural producers within irrigation modules, members of the fishing cooperatives at the Adolfo Mateos and Sanalona dams and the Mariquita dike, presidents of the local users' associations, researchers for the Agronomy Faculty of the Autonomous University of Sinaloa, and the managers of Irrigation District 010 and 074 (Aguilar, 1994, 1995, 1996, 1997).

Forty-one samples were taken during 1996 and 1997 to monitor dispersal of the weevils in the Sanalona and Adolfo Lopez Mateos dams; the Batamote, Arroyo Prieto, Hilda, and Mariquita dikes; the waste way at km 40 + 900 of the Principal Canal Humaya (CPH); and at the Andrew Weiss diversion dam. Insects were found at all points and all plants showed adult weevil feeding damage. Random sampling of waterhyacinth at Batamote, Hilda, Mariquita, and Arroyo Prieto dikes revealed the growth and expansion of the weevil population as well as their impact to this weed.

Table 3. A comparison of waterhyacinth coverage at various reservoirs in Sinaloa before and after introduction of *Neochetina* spp.

	Waterhyacinth infestation (%			
Reservoir	Area	Before weevils	After weevils*	
	(ha)	October 1994	January 1997	March 1998
Batamote Dike	134	100	65	2
Arroyo Prieto Dike	42	100	100	1
Hilda Dike	12	100	70	1
Mariquita Dike	492	80	70	20
Andrew Weiss Diversion	53	100	60	0
Adolfo López M. Dam	6393	30	20	15
Sanalona Dam	2443	20	15	4.9

^{*}Weevils were released during the period between January 1995 and August 1996.

The Mariquita impoundment was monitored to observe the impact of the weevils, but its size (482 hectares) and irregular shape made estimation of water-hyacinth coverage difficult. A similar situation occurred at the Arroyo Prieto Impoundment, where sampling was not planned but was initiated when the weevils induced an obvious reduction in water hyacinth coverage. However, these data weren't as meaningful because a base-line hadn't been established. Therefore, quantitative data are presented only for the Batamote and Hilda dikes (Figures 1 and 2).

The coverage by waterhyacinth at the Batamote impoundment was reduced considerably by the weevils. Total biomass decreased from 46,603 metric tons at the time of release to less than 1000 metric tons by December 1997. About 95% of the impoundment was covered during October 1994, whereas coverage was reduced to 2.5% by 1997.

The weevil population at Batamote impoundment fluctuated. Figure 1 shows the relationships between the biomass and coverage of water hyacinth and the insect population. Three phases were apparent. During the first phase, the weevil populations were establishing and spreading so total numbers remained low, as did the numbers per plant while the weed infestation remained severe. The plant population was reduced somewhat during the second phase but, even though it supported a large weevil population both in terms of total numbers and numbers per plant (intensity), the weed infestation remained severe. The weevil intensity attained a maximum of 7.3 adults/plant, 3.5 larvae/plant; and 1.7 pupae/plant. A declining plant population in response to this heavy attack by weevils characterized the third phase, with a concomitant decline in the overall weevil population. Even though the

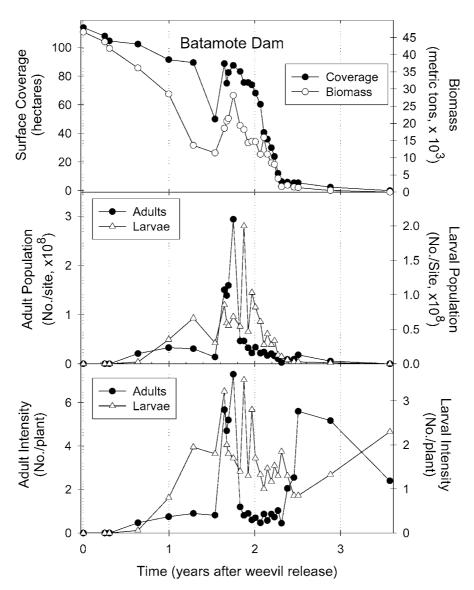


Figure 1. Graphs illustrating the decline in total waterhyacinth biomass over time at the Batamote Impoundment as related to the number of adult *Neochetina* spp. weevils per plant (top); and data on the intensities of adults, larvae, and pupae per plant over time (bottom). The x-axis represents elapsed time after release of the weevils.

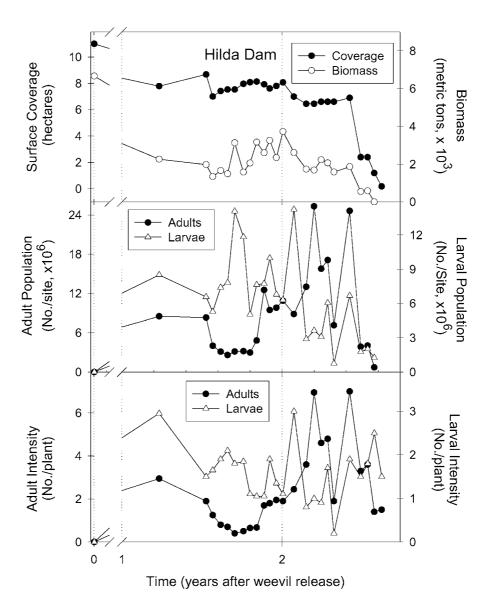


Figure 2. Graphs illustrating the decline in total waterhyacinth biomass over time at the Hilda Impoundment as related to the number of adult *Neochetina* spp. weevils per plant (top); and data on the intensities of adults, larvae, and pupae per plant over time (bottom). The x-axis represents elapsed time after release of the weevils.

total number of weevils declined from the loss of the plants during this latter phase, the intensity (numbers per plant) remained high as surviving insects converged on the few plants remaining.

Water hyacinth coverage at the 12-ha Hilda dike decreased from 100% in May 1995 (equivalent to 6,666 metric tons) to 70% during January 1997. By March 1998 only 1% of the area was infested, representing approximately 30 metric tons of waterhyacinth. The same three phases could be distinguished but they were less obvious than at Batamote. The second phase seemed longer whereas the third phase seemed shortened. Weevil intensity peaked at 7 adults/plant, 3 larvae/plant, and 0.9 pupae/plant. The relationships between water hyacinth and weevil populations are also shown in Figure 2.

The presence of the weevils and the control that they exerted on water-hyacinth was also evident at other dikes, reservoirs, and canals within Irrigation District 010 and 074 (Table 3). Results from Arroyo Prieto were notable. The surface area of this dike was completely covered during May 1995; by March 1998 waterhyacinth covered only 1% of the surface.

Mariquita is the largest dike in the Humaya system with a surface area of 492 ha. The weevils also controlled the water hyacinth here. The reduction occurred mainly during late 1997 to early 1998. Only 98.4 ha (20%) of water hyacinth remained by March 1998.

A serious waterhyacinth problem also existed on 53 ha above the Andrew Weiss head works, in the Humaya system, during 1993–1994. At that time, there was an infestation of 15 hectares (28.3%). The surface of this impoundment was completely free of waterhyacinth by March 1998.

The weevils were also widespread in the Sanalona and Adolfo Lopes Mateos impoundments. Waterhyacinth had invaded approximately 120 ha at the Sanalona dam at the entrance of the basin where it empties into the Tamazula River. Nearly all waterhyacinth plants showed signs of attack by the insects, were small, and had not produced flowers.

The implementation of biological control, particularly with *Neochetina* spp., has enhanced the operation of canals and has reduced water loss. The weevils effectively controlled waterhyacinth and were not detrimental to plants of economic or ecological importance. The density of adult weevils required to attain control was about 6 weevils/plant based on both the field cage trials and open releases (but generalizations are difficult, see Center et al., 1999a, b). Shallow sites with little water movement favored persistence of the weed, even though the plants became highly stressed and seemed near death. These mats later became submerged when water movement resumed and water levels rose.

Although these insects have been credited with controlling waterhyacinth in many other countries (Center, 1994), the reductions observed in Sinaloa

seem rapid. Julien et al. (1996) indicate that biological control can reduce waterhyacinth infestations within 3-10 years, whereas the declines noted here occurred within 2–3 years. Perhaps this was due to the care taken to release healthy weevils. This cannot be ascertained unequivocally, because comparisons with releases of diseased weevils are not possible (nor advisable). Other confounding factors may have played roles, too. The effect of the weevils, for example, was complimented by phytopathogens and, to a lesser extent, by an aquatic grasshopper *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae). Nonetheless, waterhyacinth was not being controlled until after the weevils were released. The possibility that entomopathogens may diminish the performance of introduced weed biological control agents should be seriously considered prior to the release of a new agent.

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Important cooperative interactions combined to facilitate the success of this effort. These included coordination of operational activities with research efforts, so as to allow continuation of irrigation while aiding field study; consultation with producers to identify the principle problems caused by the weed and to suggest joint projects; combined research efforts among universities, local and regional centers, and institutes; and the coordination of the National Water Commission (Comision Nacional del Agua). We sincerely appreciate the cooperation and support of the following individuals: Ing. Trinidad Contreras Morales and Ing. Juan Garcia Molina from 010 and 074 irrigation districts in Culiacan, Sinaloa; Ing Antonio Davila Capiterucho from the Conservation Division of the National Water Commission (Comision Nacional del Agua); and the farmers from the eighteen irrigation groups from the 010 and 074 districts, in Culiacan, Sinaloa.

Note

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